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14. ABSTRACT

.Results from studies conducted during this funding period provides further evidence that TBI results in neuroinflammination and altered neuroendocrine and neurobehavioral deficits that persist 72hrs following the injury. Our findings indicate that interventions aimed at modulation of the endocannabinoid (EC) system targeting degradation of 20arachidonoyl glycerlol (2-AG) and N-arachidonoyl ethanolamine (AEA) effectively reduce neuroinflammination and blood barrier leak while preserving neurobehavioral function post-TBI. Studies examined the extent to which these interventions prevent the rise in pro-inflammatory cytokine expression, neutrophil influx, blood brain barrier permeability, neurological and neurobehavioral impairments following TBI produced by lateral fluid percussion. Inhibition of EC degradation results in significant protection 24hrs post-TBI produced by lateral fluid percussion. Inhibition of EC degradation results in significant protection 24hrs post-TBO in blood brain barrier integrity, as well as significant reduction in sensory and motor damage 24-72hrs post-injury.

15. SUBJECT TERMS- lateral fluid percussion, traumatic brain injury, blood brain barrier, neuroinflammination, neurological dysfunction, endocannabinoids.

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INTRODUCTION:

Long term effects of TBI, including neuroendocrine dysregulation and neurobehavioral recovery may be ameliorated by intervention aimed at reducing short term neuroinflammation, oxidative stress, and altered neuroendocrine and behavioral functions. Our working hypothesis is that elevated levels of the endocannabinoids (EC), 2-arachidonoyl glycerol (2-AG) and N-arachidonoyl ethanolamine (AEA) should ameliorate neuroinflammatory changes following TBI. During this funding period, selective pharmacologic inhibitors have been used to decrease the degradation of 2-AG and/or AEA. Pharmacotherapy has been administered 30 min post-TBI. Studies examined the extent to which this intervention has modulated cytokine release, neutrophil influx, blood brain barrier permeability, and neurological and neurobehavioral severity impairments following TBI produced by lateral fluid percussion. Additional studies that characterize the neuroendocrine response to cardiovascular challenge after TBI have also been conducted.

BODY:

Progress Report 2nd Annual Funding Period:

Studies to date have been directed towards the completion of Milestones 1, 2, and 3 as defined in the Statement of Work. The goal of milestone 1 is to describe the impact of EC degradation inhibition on neutrophil influx, pro-inflammatory cytokine expression, oxidative injury, edema, and blood brain barrier permeability and to provide histological assessment of the protective effects of EC following brain injury. The goal of milestone 2 is to examine the effectiveness of decreasing EC degradation in maintaining neuroendocrine integrity following TBI. The goal of milestone 3 is to examine the efficacy of elevated EC levels to provide neuroprotection and improve neurobehavioral outcome as reflected in motor and cognitive function. Current efforts are focused on Tasks 1, 2, and 3.

Task 1:

Determine the effectiveness of specific inhibitors of endocannabinoid degradation in reducing neutrophil influx, pro-inflammatory cytokine expression, oxidative injury, edema, and blood barrier permeability.

a. Inflammation & oxidative stress (2h, 4h, 24h, 72h post-TBI) . Brain tissue (area of injury, prenumbral region, contralateral region, frontal cortex) content of cytokines and chemokines, oxidative stress (lipid peroxidation and catalase activity). Inflammatory cell infiltration examined by immunohistochemistry.
b. Brain edema (4h, 24h, 72h post-TBI). Wet/dry ratio determined.
c. Blood brain barrier permeability analyzed by dye tracer extravasation (24h & 72h post-TBI).
d. Cell injury by histological analysis (7d & 30d post TBI).
e. Endo-Cannabinoid Level determinations in extracted brain tissue lipids

Progress:

Efforts under Task 1 have been primarily focused towards the completion of Tasks 1a (Inflammation and oxidative stress) and Task 1c (Blood brain barrier permeability). Our studies have focused on increasing sample size for the collection and analysis of tissues at both the 24hr and 72hr time points. Additional tissues samples have been collected and analyzed for MPO myeloperoxidase activity, IL-6 protein cytokine expression, RT-PCR analysis of IL-6, IL-1, TNFα, MCP1 as well as Blood Brain Barrier

permeability. It is important to note that a different set of animals is required for doing analysis for each of the tasks outlined in Task 1a-d. Future efforts will be directed towards analyzing brain tissue EC levels and to establish the efficacy of the enzymatic inhibitors in maintaining elevated 2-AG and AEA levels (Task 1e). Additionally, fixed brains for histological analysis using hematoxylin-eosin staining will be evaluated to determine the extent of cortical tissue damage following traumatic brain injury (Task 1d).

Summary of findings:
Task 1a

Inhibition of Endocannabinoid (EC) Degradation Following Traumatic Brain Injury Decreases Neutrophil Influx

TBI induced by lateral fluid percussion (1.8 atm; ~ 25ms) delivered to the dura resulted in an increase in brain myeloperoxidase (MPO) activity in the ipsilateral region of injury as compared to the sham animals post injury (Fig.1a-c).Treatment with the selective EC inhibitor JZL184 resulted in markedly decreased MPO activity in the ipsilateral brain region when compared to the TBI/Vehicle treated animals at 24 and 48hrs post-TBI. However this protective effect was not evident at the 72 hour time point. In contrast, MPO activity measured in brains of animals treated with the selective EC inhibitor URB597 did not show effective suppression in neutrophil influx as our data indicates no differences between that of the TBI/Vehicle treated animals.

Our findings imply higher efficacy of JZL184 in attenuating the inflammatory response early on, but also indicate a relatively short-lived anti-inflammatory effect, as by 72hr MPO values are elevated. This suggests the possibility that additional doses of the EC degradation inhibitors may prove beneficial at enhancing the neuroprotective outcomes from TBI. Studies are currently underway to prolong the duration of drug treatment to establish the optimal period of intervention by administrating a second dose of the selective EC enzyme degradation inhibitors 24hrs post TBI.

Fig: 1a

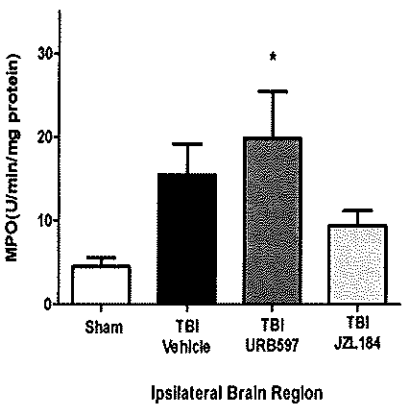

Fig: 1b

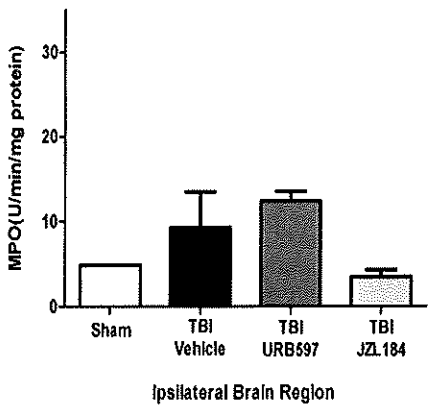

Fig: 1c

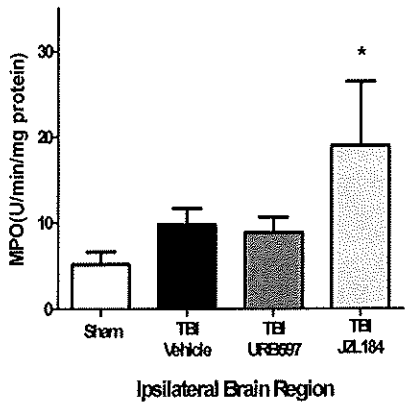

Figure 1a-c. Myeloperoxidase (MPO) Activity (U/min/mg protein) in the Ipsilateral Brain Region Excised at 24hr, 48hr and 72 hrs post-TBI. Inhibition of E.C. degradation proves to be effective at decreasing MPO activity 24hr and 48hrs following TBI when treated with the selective inhibitor JZL184 (16mg/kg, IP, 30minutes post-TBI)
Sham; TBI/Vehicle (alcohol, emulphor, saline 1:1:18); TBI/JZL 184 (16mg/kg, IP); and TBI/URB 597 0.3mg/kg, IP) (n=6/group); P*<0.0001 compared to Sham; Analyzed by One-way ANOVA

Effects of Inhibiting Endocannabinoid Degradation in Reducing pro-Inflammatory Cytokine Expression Following Traumatic Brain Injury

Traumatic Brain Injury initiates a neuroinflammatory cascade characterized by an increased production of proinflammatory cytokines and chemokines, such as interleukin IL-1, IL-6, tumor necrosis factor (TNFα) and MCP1. In the case of TBI, this complex neuroinflammatory cascade can promote neuroinflammation and potentially lead to neurodegeneration. We have previously demonstrated that preventing degradation of 2-arachidonoyl glycerol (2-AG) and N-arachidonoyl-ethanolamine (AEA) ameliorate the neuroinflammatory response at 24hrs post-TBI.

We analyzed our 24hr and 72hr tissue samples post-TBI for IL-6 activity using an Invitrogen IL-6 Rat Elisa Kit. Current studies indicate no significant difference between Sham and TBI/Vehicle treated animals (Fig. 2a-b). Furthermore, drug interventions (JZL184 16mg/kg, IP, 30min's post-TBI) and (URB597 0.3mg/kg, IP, 30 min's post-TBI) did not effectively decrease IL-6 expression as anticipated. The lack of significant differences in tissue expression of IL-6 will be further examined. It is possible that the sensitivity of the current analytical approach may preclude us from detecting differences in tissue cytokine levels. We are currently evaluating advanced technology for cytokine detection in tissues that may lead to conclusive results. Current efforts are directed towards optimizing a new method to evaluate these cytokines for improved accuracy and sensitivity.

Fig: 2a

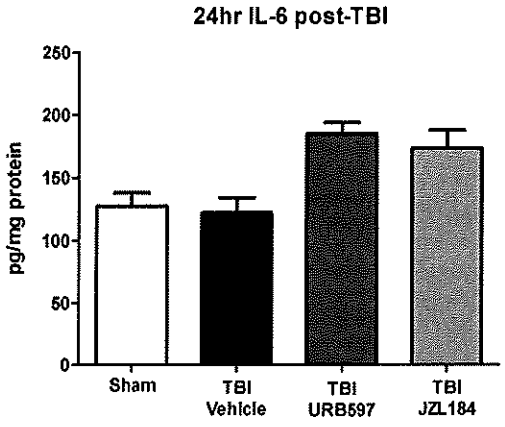

Fig: 2b

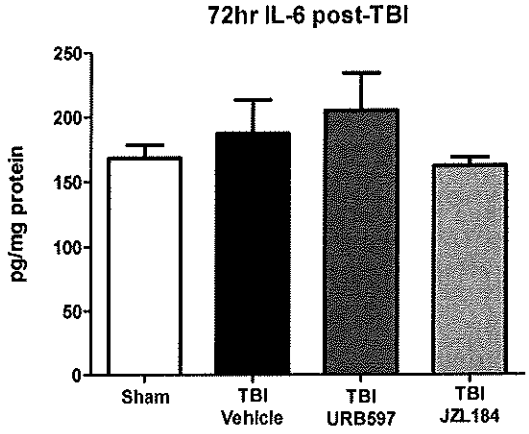

Fig 2a-b: IL-6 Activity (pg/mg protein) Measured in the Ipsilateral Brain Region Excised at 24hr and 72hrs post-TBI.

Inhibition of 2-AG and AEA degradation by the use of the selective inhibitors (JZL184; 16 mg/kg and URB597 0.3 mg/kg, respectively, IP) administered 30 min post-TBI does not appear to reduced the proinflammatory cytokine IL-6 when compared to vehicle treated animals. Measured byIL-6 Rat ELISA (n=6/group).

To provide a more sensitive measure of the proinflammatory response following TBI we measured cytokine mRNA expression by RT-PCR analysis. Data for the 72 h post-TBI indicates that MCP1 is the only upregulated cytokine in TBI animals (Fig: 3b). Interestingly IL-6 and IL-1 expression are significantly upregulated only in the JZL 184 treated animals (Fig: 3c, 3d). These findings are consistent with our MPO results seen at the 72hr time point (Fig: 1c). This paradoxical increase in expression of IL-1 and IL-6 in the group of animals that showed superior initial reduction in MPO remains to be further explored. Studies are currently underway to repeat drug treatment by administrating a second dose of the selective EC enzyme degradation inhibitors 24hrs post TBI. We hypothesize that additional administration of the EC breakdown inhibitors will produce a significant reduction in proinflammatory response at these later time points. Moreover, additional samples are currently in queue for RT-PCR analysis at the 24hr time point.

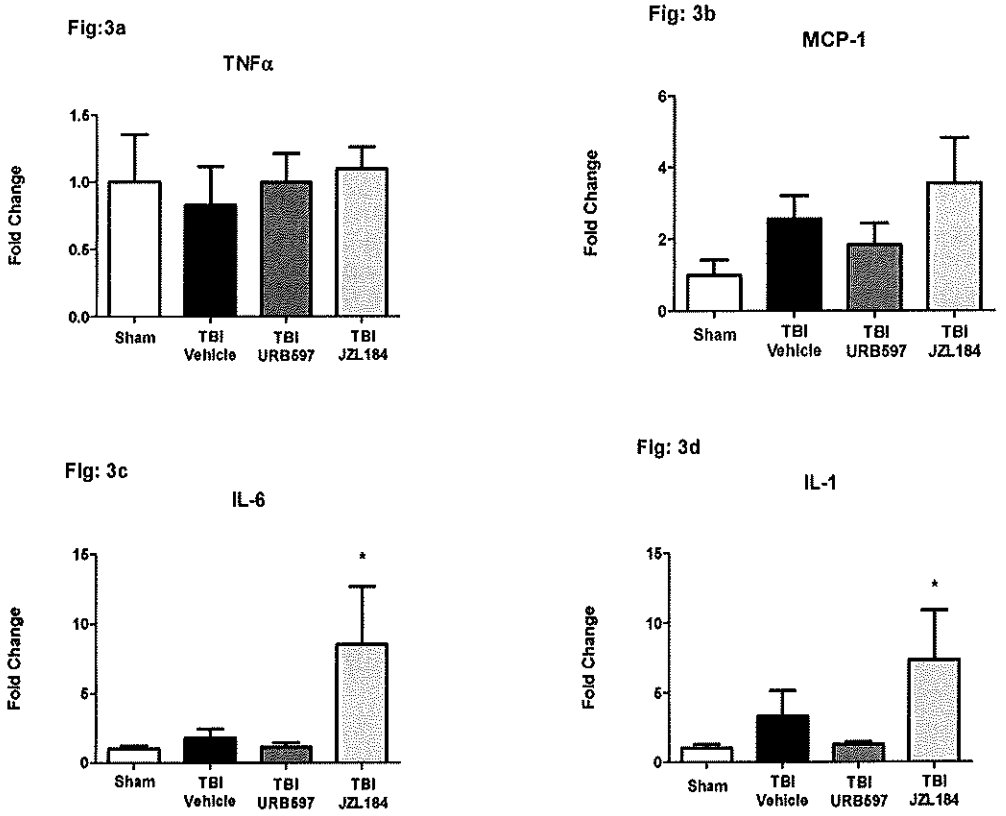

Fig: 3a-d Effects of Inhibiting Endocannabinoid Degradation in Reducing pro-Inflammatory Cytokine Expression 72hrs Following Traumatic Brain Injury.

Brain tissues were analyzed for TNFα, MCP1, IL-6 and IL-1 by RT-PCR 72 hrs post injury in Sham; TBI/Vehicle (alcohol, emulphor, saline 1:1:18); TBI/JZL 184 (16mg/kg, IP); and TBI/URB 597 0.3mg/kg, IP) treated animals. (n=6/group); P* < 0.0001 Analyzed by One-way ANOVA

Task 1c

Inhibition of Endocannabinoid Degradation Reduces Blood Brain Barrier Dysruption Following Traumatic Brain Injury.

To date, we have examined the effectiveness of inhibiting endocannabinoid enzymatic degradation on blood brain barrier integrity at the 24h and 72h time points using Evan's Blue dye tracer extravasation. TBI alone results in a significant increase in blood brain barrier leak (~2-fold) as compared to the sham animals (Fig: 4). Results from this study suggest that inhibition of 2-AG and AEA degradation using (JZL184; 16 mg/kg and URB597 0.3 mg/kg) administered IP, 30 min post-TBI improves blood brain barrier integrity in the targeted ipsilateral brain region of injury when compared to the TBI/Vehicle group. Specifically, animals treated with the selective inhibitor JZL184 sacrificed 24hrs post TBI experienced significant ($p\$<0.05$) improvements in blood brain barrier integrity when compared to the vehicle treated animals (Fig 4). The compromise in blood brain barrier structure does not appear resolved 72hr post injury as seen in the TBI/Vehicle animals without the use of EC drug interventions. While not statistically significant, both JZL184 and URB597 are effective at minimizing blood brain barrier leak in the ipsilateral region 72hrs after injury (Fig 4). We anticipate that repeated dosing of the EC inhibitors may produce greater protection, and result in marked blood brain barrier resolution at the later time-points. These efforts are currently in process.

Fig: 4

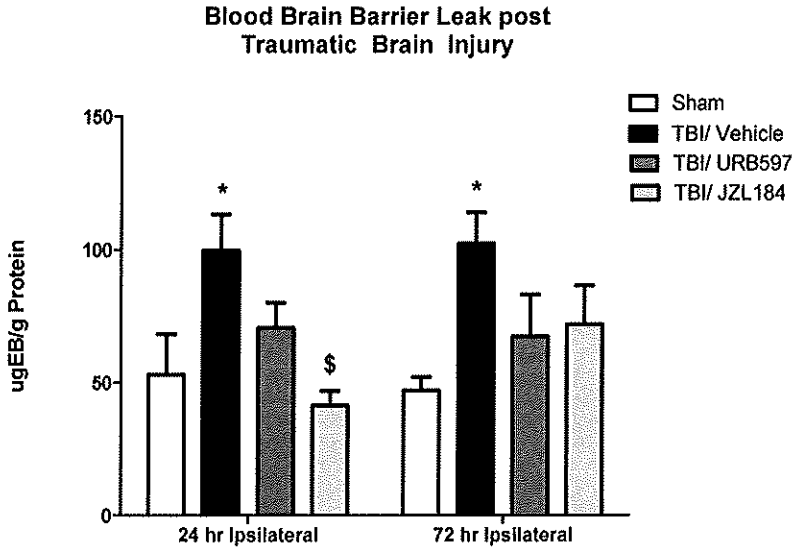

Figure 4: Inhibition of EC degradation reduces blood brain barrier breakdown 24h and 72h post-TBI. Traumatic Brain Injury significantly disrupts the integrity of the blood brain barrier when compared to the sham uninjured animals. Inhibition of 2-AG and AEA degradation with the use of the selective inhibitors (JZL184; 16 mg/kg and URB597 0.3 mg/kg, respectively, IP) administered 30 min post-TBI helps to reduced blood brain barrier dysfunction when compared to vehicle treated animals. 24hrs post-TBI animals treated with JZL184 is significantly different than that of the TBI/Vehicle treated animals. $P^*<0.05$ compared to sham; $P\$<0.05$ compared to TBI/Vehicle; Analyzed by Two-way ANOVA (n=5-19/group)

Task 2:

Determine the effectiveness of the selective increase in endogenous 2-AG and AEA levels in preventing neuroendocrine dysfunction following TBI.

a. Basal unstimulated neuroendocrine function
b. Autonomic and neuroendocrine response to cardiovascular challenge
c. Autonomic and neuroendocrine response to water deprivation test

Progress:

Summary of findings:

Continuing efforts are directed towards collecting data under Task 2a, basal unstimulated neuroendrocrine function. Samples are being collected from vehicle and drug treated animals for analysis of corticosterone levels in blood plasma at the 2hr, 4hr, 24hr and 72hr time point's following TBI. Collection of blood samples past the initial 24 h time point has been a challenge in some animals, as the indwelling catheters have a high rate of failure at that time.

Blood pressure and heart rate measurements are recorded immediately prior to, during, and after TBI in 30 second intervals. Results to date indicate a significant decrease in heart rate immediately following TBI when compared to the un-injured sham animals (Fig. 5). No significant alteration in mean arterial blood pressure has been observed during the immediate and early time post-TBI. However, it is important to note that animals are lightly anesthetized with 3% isoflurane during the time of injury, and that may explain the lack of significant modulation of blood pressure response

Fig: 5

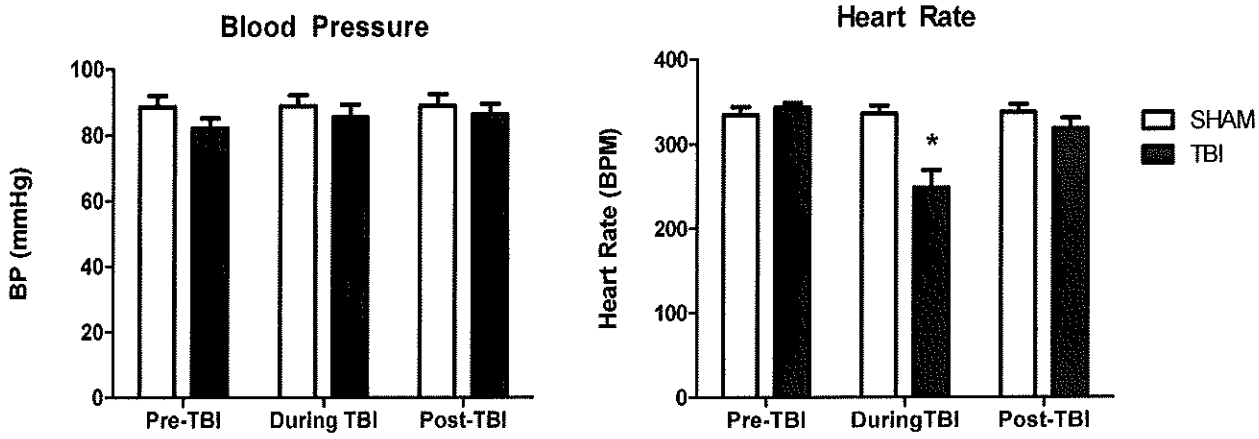

Figure 5: Blood Pressure (mmHg) and Heart Rate (BPM) response to TBI.
All animals were anesthetized using 3% isoflurane and were continuously monitored via the arterial catheter connected to a pressure transducer for changes in blood pressure and heart rate using the LabPro System. Blood pressure and heart rate were recorded and analyzed for a period of 30 seconds at each of the following time point intervals (pre-TBI, during TBI, post-TBI). Results to date indicate significant heart rate changes during the time of the traumatic brain injury. (n=18/Sham; n=50/TBI).
P * <0.01 Sham vs. TBI; Analyzed by Two-way ANOVA

Characterization of Neuroendocrine and Cardiovascular Alteration Following Traumatic Brain Injury

Additional efforts have been made to characterize the model for determining the effectiveness of the selective increase in endogenous 2-AG and AEA levels in preventing neuroendocrine dysfunction following cardiovascular system challenge post-TBI (Task 2b). To date, we have examined the neuroendocrine and cardiovascular system response in sham vs. TBI animals 24 hrs post-TBI when challenged with hydralazine HCl (10mg/kg, administered IV).

To reveal the extent of subclinical neuroendocrine dysregulation following TBI, animals were subjected to a pharmacological challenge to induce hypotension. Mean arterial blood pressure (MABP) was recorded from TBI and sham animals only. TBI animals present with lower baseline MABP as compared to un-injured sham animals (Fig 6a) p= NS. After 15 min of stable blood pressure recordings, hydralazine hydrochloride (10 mg/kg) was slowly injected intravenously through the jugular catheter. Mean arterial blood pressure decreased substantially (~50% of baseline) within 10 min of hydralazine HCl injection, and remained suppressed during the monitoring period of 90 minutes. Initial results suggested that there appeared to be a trend for more persistent hypotension in the TBI animals vs. sham animals. This study was repeated to include a more frequent time point for data collection, to ensure that any early differences in response were not missed by the initial set of studies. The results showed that the hydralazine-induced decrease in blood pressure was similar in sham and TBI animals (Fig 6b).

While preliminary findings suggest that hydralazine appropriately lowers MABP, it does not appear to unmask neuroendocrine dysregulation of the cardiovascular system. Sodium nitroprusside has a rapid onset of action. We proceeded to challenge the animals with sodium nitroprusside following the same protocol described above for hydralazine. Post-TBI animals challenged with sodium nitroprusside (10mg/kg) intravenously through the jugular catheter appear to demonstrate dramatic reductions in MABP seen at 30 seconds post injection coupled with slower and incomplete recovery to baseline compared to time-matched sham controls (Fig 6c). These findings suggest that integrity of cardiovascular regulation may be compromised following TBI, and that the use of sodium nitroprusside, as compared to hydralazine hydrochloride, may be of more use in unmasking cardiovascular dysregulation. Additional studies will determine if this is a reproducible finding. Furthermore, the use of hemorrhagic sock as a challenge to produce the decrease in mean arterial blood pressure will be considered. Studies including inhibitor-treated TBI animals are yet to be performed.

Fig: 6a

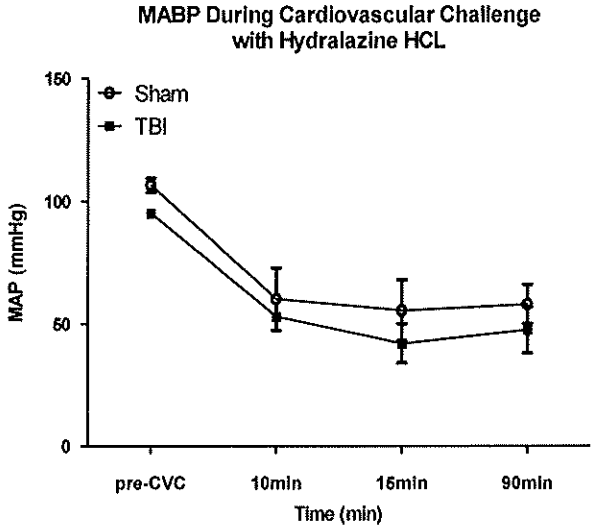

Fig: 6a. MABP in response to Cardiovascular Challenge with Hydralazine HCL (10mg/kg).

Animals subject to TBI appear to have a lower baseline mean arterial pressure (MABP) 24 hours post-TBI compared to sham. These results were obtained from preliminary studies performed to identify the optimal dose of hydralazine for the test. (Sham n=3; TBI n=3).

Fig: 6b

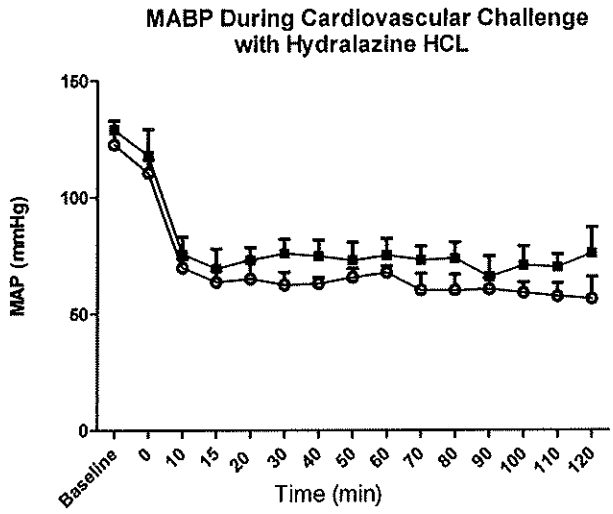

Fig: 6b. MABP in response to Cardiovascular Challenge with Hydralazine HCL (10mg/kg).

Both TBI and sham animals showed similar decrease in MABP following hydralazine HCl, suggesting intact cardiovascular counteregulatory responses post-TBI. (Sham n=9; TBI n=9)

Fig: 6c

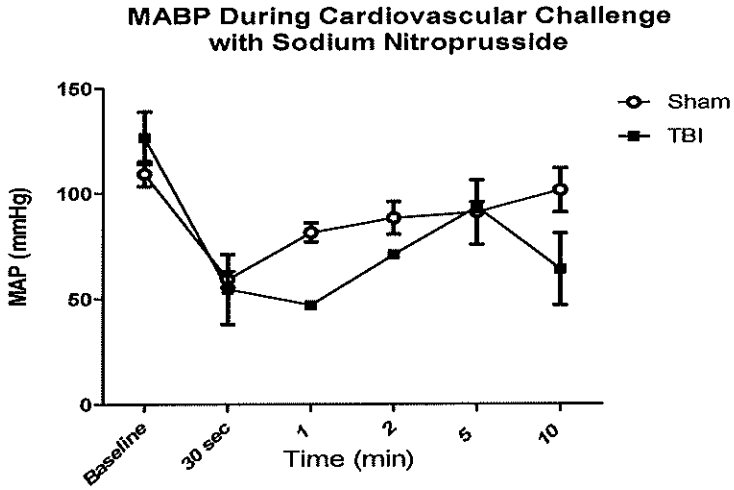

Fig: 6c. MABP During Cardiovascular Challenge with Sodium Nitroprusside (10mg/kg).

In response to sodium nitroprusside, post-TBI animals demonstrated more dramatic reductions in MAP as well as slower recovery to baseline as compared to time matched sham controls. (Sham n=3; TBI n=3).

Task 3:

Determine the capacity of increased EC levels to protect neurobehavioral and cognitive function following TBI.

a. Severity of TBI determined by the righting reflex.
b. Sensory reflex examined by the forelimb and hind limb reflex.
c. Somatomotor function examined by a beam balance task and beam-walking task.
d. Cognitive function tested by the radial-arm maze.

Progress:

Summary of findings:

Efforts have been directed towards the completion of Tasks 3a, 3b, 3c, and 3d. The severity of injury is determined by the amount of time it takes the animal to right itself following the injury. This measure reflects a loss of consciousness following the injury and is used as a point of reference for neurological and neurobehavioral assessments post TBI. All animals are first placed in a 3% isoflurane (pre-charged) anesthesia induction chamber. Once anesthetized, each animal is then placed into the stereotaxic frame and fitted with the cranial female luer loc connected to the lateral fluid percussion (LFP) system. Although the animals are not actively receiving isoflurane at the time of injury, they are still anesthetized. Upon the return of a hind-limb toe pinch response, the traumatic brain injury (1.8 atm; ~ 25ms) is delivered to the dura. Following the injury, the animal is removed from the stereotaxic frame and placed on its right side to recover. The time it takes for the animal to regain complete consciousness is recorded. Sham control animals are connected to the LFP system in the exact same manner, but not subjected to the TBI. Our results to date indicate that TBI produces a significant delay in righting reflex time when compared to the uninjured sham animals ($p^*<0.0001$) (Fig.7). Because the inhibitors are administered following TBI, this outcome measure is not expected to be affected by the drugs.

Fig: 7

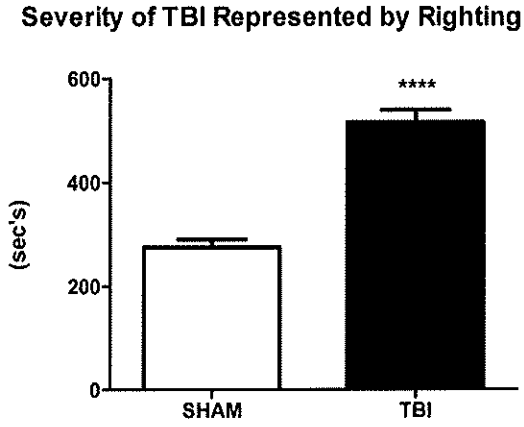

Figure 7: Severity of TBI Represented by Righting Reflex time Immediately post TBI.

Animals subject to traumatic brain injury have a significantly increased duration of loss of consciousness determined by the time it takes the animal to right itself when compared to the sham uninjured animals. P * value =0.0001 Sham vs. TBI analyzed by a 2-tailed unpaired T-test.

Ongoing studies have focused on assessing neurological and neurobehavioral outcomes primarily at the 24 and 72 hour time points following TBI. Neurological (NSS) and Neurobehavioral (NBS) function is examined by a combination of somatomotor and cognitive assessments. The extent of neurological injury consists of a 25 point maximum scale comprised of tests to evaluate: motor function (spontaneous or enticed), somatomotor function (balance beam, beam walking, and ability to stand on an inclined plane), somatosensory function (proprioception, and pain response by Hargreaves' Method) and cognitive function (radial arm maze, open field, and acoustic startle response). The degree of neurobehavioral injury consists of a 12 point maximum scale which evaluates stereotypical behavior (such as grooming, exploratory activity, and novel object recognition). Animal behavior is scored based on their ability to carry out the above mentioned tasks; zero being normal while an increased score represents the level of impairment. Each animal is trained in the tasks prior to the traumatic brain injury, and performance during this time is used as a reference control. Following the traumatic brain injury, each animal is re-assessed at the 2h,24h,48h and 72h time points to determine the degree of neurological and neurobehavioral dysfunction. The selective Endocannabinoid enzyme degradation inhibitors (JZL184; 16 mg/kg and URB597 0.3 mg/kg, IP) are administered 30 minutes post traumatic brain injury. We hypothesized that administration of the EC breakdown inhibitors would produce a significant reduction in inflammatory and oxidative tissue damage, therefore improving neurological and neurobehavioral outcomes.

Our studies have demonstrated that TBI leads to a marked increase in NSS and NBS scores which is evident in all groups as early as 2 hours post-TBI (Fig. 8 and Fig. 9). Specifically, in the TBI/Vehicle treated group, the increased NSS and NBS scores remain elevated throughout the entire 72hr course of the study when compared to the sham uninjured animals ($p^{*} < 0.0001$). While NSS and NBS scores 2 hours post-TBI were not different between vehicle-treated, JZL184, and URB597 treated TBI animals, results from the 24, 48, and 72 hour time points suggest that the selective inhibitor JZL184 is extremely effective at improving neurological and neurobehavioral severity scores. Animals treated with the selective inhibitor JZL184 assessed 24 and 48hrs post TBI experienced significant improvements in neurological outcomes when compared to the vehicle treated animals ($p\$<0.05$) and JZL184 significantly improved TBI outcomes when compared to the other selective inhibitor URB597 ($p\#<0.01$). Animals treated with URB597 post-TBI showed little improvements in NSS or NBS scores when compared to the TBI vehicle treated animals over the 72hr course of the study.

As previously reported, the observed increased NSS and NBS scores following TBI at the 2hr time point in the drug treated groups might suggest that early post-TBI treatment with the EC enzyme inhibitors could be associated with a marked increase in local concentrations of EC, in turn, obscuring our data interpretation. Reports in the literature indicate that these doses have been shown to increase EC levels in the brain (8-fold) for up to 12hrs following treatment and to produce analgesic effects. Recent studies in house have confirmed that these increased scores are indeed due to the combination of TBI with the additional EC treatment that is leading to the observed behavioral effects seen 2hrs post injury. When the EC inhibitors are administered to uninjured sham animals, no behavioral side effects were observed in either NSS or NBS assessments, as these animals exhibited completely normal behavior.

Neurological Severity Score post-TBI When Treated with EndoCannabinoid Inhibitors

Fig: 8

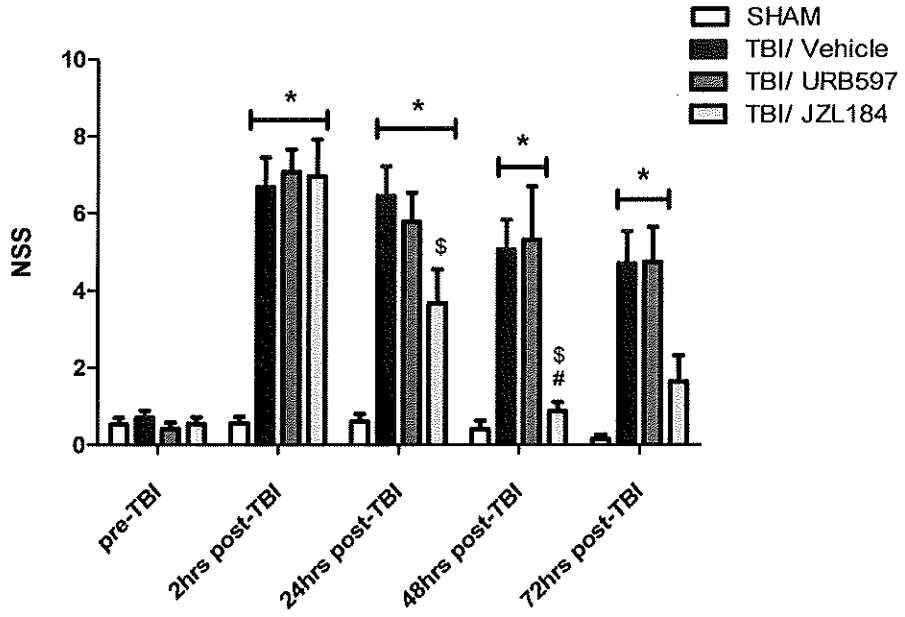

Figure 8: Neurological Severity Score (NSS) Following Fluid-Percussion Induced Traumatic Brain Injury.
These scores represent the degree of neurological dysfunction following traumatic brain injury at the following time points: 2hr, 24hr, 48hr and 72 hrs. NSS scoring (0=normal; 25=severe)
Sham (n=33); TBI vehicle (n=51); TBI URB 597 (n=31) and TBI JZL 184 (n=26)
P * value < 0.0001 vs. sham; P $ value < 0.05 JZL 184 vs. Vehicle; P # value <0.01 JZL184 vs. URB597

NeuroBehavioral Severity Score post-TBI When Treated with EndoCannabinoid Inhibitors

Fig: 9

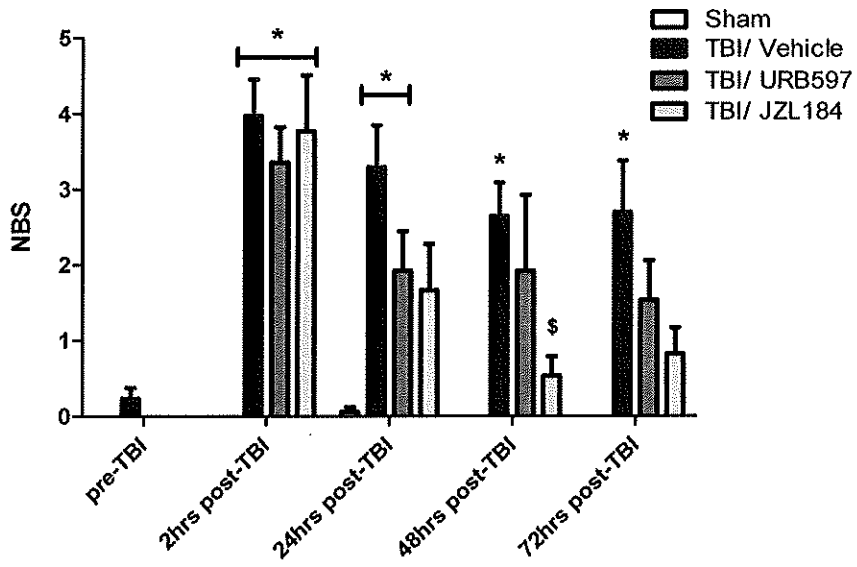

Figure 9: NeuroBehavioral Severity Score (NBS) Following Fluid-Percussion Induced Traumatic Brain Injury.
These scores represent the degree of neurobehavioral dysfunction following traumatic brain injury at the following time points: 2hr, 24hr, 48hr and 72 hrs. NBS scoring (0=normal; 12=severe)
Sham (n=33); TBI vehicle (n=51); TBI URB 597 (n=31) and TBI JZL 184 (n=26)
P * value < 0.05 compared to sham; P $ value < 0.05 JZL184 vs. Vehicle
Analyzed by Two-way ANOVA

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstrated that inhibition of EC degradation can limit pro-inflammation responses immediately following TBI
- Demonstrated that inhibition of EC degradation can significantly maintain blood brain barrier integrity following TBI
- Endocannabinoid degradation inhibition effectively improves neurological and neurobehavioral outcomes following TBI.
- A single dose JZL184 administered 30 minutes following injury is superior to URB597 in improving tissue markers of inflammation (MPO), brain structural damage (BBB), neurological (NSS) and neurobehavioral (NBS) outcomes following TBI.

REPORTABLE OUTCOMES:

Publications: N/A

Presentations: N/A

Conclusion:

The results from our ongoing studies during this funding period have provided further evidence that the selective pharmacologic inhibitor of E.C. degradation JZL184 is effective at reducing tissue markers of inflammation and brain structural damage such as blood brain barrier disruption as early as one day post-TBI. However, the neuroprotective effects reported thus far appear short lived, and limited to the 72hr period post-TBI. Additional studies will be performed in an effort to investigate whether these improved early outcomes can persist for longer periods of time such as one week – one month post injury. Attenuation of the acute increase in pro-inflammatory cytokines and chemokines following TBI should decrease neurologic injury and improve functional outcomes. Studies are currently underway to repeat drug treatment to establish the optimal length of intervention. This will be accomplished by administrating a second dose of the selective EC enzyme degradation inhibitors 24 hrs post TBI.

Continuing studies will further provide evidence that EC modulation is a potential therapeutic treatment and can target the underlying pathophysiology of TBI while decreasing functional deficits. Additional efforts are directed towards analyzing brain endocannabinoid levels to establish the efficacy of the enzymatic inhibitors in maintaining elevated 2-AG and AEA levels. Furthermore, brains for histological analysis using hematoxylin-eosin staining will be harvested to evaluate the extent of cortical tissue damage following traumatic brain injury. Cerebral edema due to neuroinflammation is a major factor in the neurologic morbidity associated with TBI; therefore brain wet/dry ratio will also be determined to evaluate water content in the tissue.

REFERENCES: N/A
APPENDICES: N/A